

# NON-NATURAL AMINO ACID REPLICATION-DEPENDENT MICROORGANISMS AND VACCINES

## CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of Ser. No. 13/120,255, filed on Apr. 25, 2011, which is a national phase entry in the United States under 35 U.S.C. § 371 from International Application Number PCT/US09/58668 filed on Sep. 28, 2009, which is incorporated by reference herein in its entirety and claims the benefit of priority to U.S. provisional patent application Ser. No. 61/100,688 filed on Sep. 26, 2008, the specifications and disclosures of which are incorporated herein in their entirety for all purposes.

## FIELD OF THE PRESENT INVENTION

The invention pertains to vaccines. In some embodiments, the present invention pertains to compositions and methods of producing vaccines, including whole organism vaccines, with limited or no replication abilities through the use of non-natural, unnatural, or non-naturally encoded amino acids.

## BACKGROUND OF THE PRESENT INVENTION

Until now, there has been relatively slow technical progress and even more than 100 years after Louis Pasteur passed away, his "III" (Isolation, Inactivation, Injection) protocol continues to be applicable. With the advent of molecular biology and recombinant protein technology, however, people have started to develop subunit vaccines and genetically engineered whole-organism-like vaccines. More recently, advances in immunology have made several classes of immunopotentiators available, including Toll-Like Receptor (TLR) ligands. Generally, newer generation vaccines and their adjuvants have been becoming more and more well defined chemically and genetically.

The development of therapeutics and in particular vaccines directed against pathogens such as viruses, bacteria, protozoans, fungi is ongoing. Such research has proved invaluable in preventing the spread of disease in animals including humans. In fact, in modern medicine, immunotherapy including vaccination has eradicated smallpox and virtually eradicated diseases such as polio, tetanus, tuberculosis, chicken pox, and measles.

Generally, ideal vaccines have a long shelf life, are capable of inducing long lasting immunity against a pre-selected pathogen and all of the phenotypic variants, are incapable of causing the disease to which the vaccine is directed against, are effective therapeutically and prophylactically, are easily prepared using economical standard methodologies and can be administered easily in the field.

There are four major classes of commercially available vaccines. They include non-living whole organism vaccines, live attenuated vaccines, vector vaccines, and subunit vaccines. Vaccination with non-live materials such as proteins generally leads to an antibody response or CD4+ helper T cell response while, vaccination with live materials (e.g. infectious viruses) generally leads to a CD8+ cytotoxic T-lymphocyte (CTL) response. A CTL response is crucial for protection against pathogens like infectious viruses and bacteria. This poses a practical problem, for the only certain way to achieve a CTL response is to use live agents that are

themselves pathogenic. The problem is generally circumvented by using attenuated viral and bacterial strains or by killing whole cells that can be used for vaccination. These strategies have worked well but the use of attenuated strains always carries the risk that the attenuated agent may recombine genetically in the host and turn into a virulent strain. Thus, there is need for therapeutics and methods that can lead to CD8+ CTL response by vaccination with non-live materials such as proteins in a specific manner.

Subunit vaccines have provided one means for dealing with some of these problems. Such vaccines generally comprise a sub-cellular component derived from a pathogen of interest. A subunit component can be either produced from a defined sub-cellular fraction of the pathogen, be a purified protein, nucleic acid or a polysaccharide. All of these elements have an antigenic determinant capable of stimulating an immune response against the pathogen of interest. Generally, the sub-cellular component of the subunit vaccine is obtained either by purifying a preparation of disrupted pathogen or synthesised using well-known procedures.

There are, however, several limitations associated with subunit vaccines. First, a requirement for the production of such a vaccine is that the antigenic determinant(s) must be characterised and identified. This imposes limitations on their use, particularly against highly variable antigenic determinants. Second, subunit vaccines are generally ineffective in stimulating cytotoxic T cell responses. Third, the immunity conferred by subunit vaccines is often short lived and therefore requires continual booster injections. Very few recombinant expressed subunit vaccines have been shown to induce strong and long lasting immunity in vaccinated animals (including man). One notable exception is the recombinant surface antigen Hepatitis B vaccine used in man. One of the problems associated with the use of such vaccines appears to be in correctly presenting the antigens to the immune system such that strong humoral immunity and strong cell-mediated immunity are induced. In particular, existing recombinant (subunit) vaccines do not appear to result in strong 'memory' responses such that vaccinated animals react very quickly when they are exposed to natural infections caused by a pathogen.

By way of example only, deficiencies in current subunit vaccines prepared from pestiviruses like bovine viral diarrhoea virus (BVDV) have been extensively reported. These studies have shown that even though large amounts of recombinant protein were used in the vaccines, there were poor protection rates seen showing that the vaccines failed to protect from challenge with live BVDV isolates (either homologous protection or heterologous protection).

There are many infectious diseases for which an effective vaccine has not yet been developed, and many of the currently available vaccines provide only partial protection against disease. Further, there are gaps in the vaccine field. Live vaccines produce stronger, broader, and more durable immunity than other types of vaccines. There is a need for a safer live vaccine vehicle, which will be unable to cause disease even in immunosuppressed individuals. There is also a need for vaccines that induce cell-mediated immunity and not just antibody-based immunity. And, there is a need to induce protective immune responses directly at the mucosal surfaces of the body, where most pathogens gain entry. Thus, there is a need for improved vaccines. The present invention seeks to provide an improved therapeutic vaccine which ameliorates at least some of the disadvantages over existing prior art.